Lipid Nanoparticles for Reviving Antibiotics: Efficacy of a Gel of Daptomycin in a Methicillin-Resistant Staphylococcus aureus Rabbit Osteomyelitis Model

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ABSTRACT

Background: Daptomycin (DAP) is a bactericidal antibiotic with activity against Gram-positive organisms, including methicillin-resistant Staphylococcus aureus (MRSA) isolates, but its administration is exclusively by route. Lipid Nano-Particles (LNC) are known to vehicle medicines and could offer some formulation options. The efficacy of LNC-daptomycin (LNC-DAP) formulated in a gel was compared with that of other antibiofilm drugs in a MRSA osteomyelitis rabbit model. Methods: Femoral trepanation of rabbits was performed, followed by injection of 30 µL of S. aureus suspension into the bone cavity. A surgical debridement of the infected tissues was performed 3 days later and animals were randomly assigned to a treatment (control, LNC-DAP [application of 50 mg/L (Low Dose) or 200 mg/L (High Dose)], Intravenous (iD), Simvastatin (SIM), VAN, DAP) or a sham treatment group (iD, SIM, VAN, DAP). RESULTS: The anti-biofilm activity was assessed on biofilm growth over 4 days. A rabbit gel with LNC-daptomycin (LNC-DAP) showed significant better biofilm reduction as compared to iD antibiotic regimen. Conclusions: In this model, a gel with lipid nano-encapsulated daptomycin (LNC-DAP) showed significant anti-biofilm activity in vivo and prolonged therapeutic effect. The use of LNCs for local delivery of antibiotics is a promising approach to treat biofilms or to develop new antibiofilm agents.

INTRODUCTION

Biofilm and joint infections (also called osteoarticulare infections, OAI) are particularly affected by the role of multiple-resistance bacteria. In addition, these infections are particularly difficult to treat due to local conditions (ischemic areas, bacteria sequestration in bone tissue, acidic pH, enzymes, calcium, etc.) and biofilm production (extracellular matrix), which decrease the efficacy of antibiotics administered through systemic delivery. Daptomycin (DAP) is a bactericidal antibiotic with activity against Gram-positive organisms, including methicillin-resistant Staphylococcus aureus (MRSA) isolates, but its administration is exclusively by route. Lipid Nano-Capsules (LNCs) are known to vehicle medicines and could offer some therapeutic options.

The aim of this work was to compare the efficacy of LNC-daptomycin (LNC-DAP) formulated in a gel with that of other antibiofilm drugs in a MRSA osteomyelitis rabbit model.

On day 0, we used a percutaneous transplantar approach to perform a femoral trepanation of the right knee using a hand held bone marrow deep needle (9 gauge) under general anaesthesia (ketamine, 10 mg/kg iv; and xylazine, 1 mg/kg iv). The skin incision was created between the two femoral condyles through the epiphysis, physis and metaphysis to reach the medullar canal. Following needle removal, the skin incision was closed. A bacterial suspension of 1 mL of S. aureus adjusted to 10^9 CFUs was injected into the knee cavity. Infection was allowed to develop for 4 days, and then a surgical debridement of the infected tissues was performed followed by an articular wash using 50 mL of 0.9% saline buffer. Samples of bone marrow and bone were removed, placed immediately on ice, weighed, homogenized in 0.5 mL of saline buffer, and then spread on agar plates using a spiral system. Treatment was started 72 h after inoculation, and antibiotics were administered for a 4 day course. At the end of the 4 day regimen, animals were euthanized, and epiphyseal bone samples and femoral bone marrow were removed. Diations at 10^10 and 10^8 were performed to eliminate potential carry-over effects. Bacterial counts were determined after 48 h of incubation at 37 °C. The efficacy measurement was made by comparing the bacterial load before (day 0 after infection) and after (day 7 after infection) antibacterial therapy.

Therapeutic regimens:
- Local administration of LNC-daptomycin (LD) and high (HD) doses.
- iD treatment (human-equivalent dose of 30 mg/kg/given once daily).
- iv-cloxacillin (human-equivalent dose of 600mg/d2).

DISCUSSION / CONCLUSIONS

- The nanotechnology manufacturing process of daptomycin fabricated with high entrapment efficiency (100%).
- A rabbit pharmacokinetic study highlighted a brief plasmatic and tissue distribution with persistence of high concentrations of daptomycin both in bone and bone marrow up to 4 days after one application of LNC-DAP.
- LNC-DAP implanted into rat femoral condyle defects is well tolerated in terms of weight, macroscopic observations of tissues (no lesion of the heart, the lungs, the liver, the spleen, the kidneys was observed) and bone histologic analyses (no acute toxic effect at the macroscopic level), related to the presence of the gels was observed in any animal at the time of 4 days following implantation.
- A gel with lipid nano-encapsulated daptomycin (LNC-DAP) showed significant in vivo activity after one topical application in comparison with majors anti-staphylococcal drugs administered by IV route for 4 days in a MRSA osteomyelitis rabbit model.
- The use of LNCS for local delivery of antibiotics is a promising approach to revive old antibiotics or to develop new antibacterial agents with solubility issues for example.